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TECHNICAL MANUSCRIPT 71

A MOBILE LABORATORY UNIT FOR EXPOSURE OF ANIMALS AND HUMAN VOLUNTEERS TO BACTERIAL AND VIRAL AEROS

JUNE 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

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Erratum

To: Recipients of Technical Manuscript 71
From: Chief, Editorial Branch, Technical Information Division

Literature Cited item 9 on page 25 of Technical Manuscript 71 is in error. Please substitute the following citation:

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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

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William Robert Griffith

AEROBIOLOGY DIVISION

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ABSTRACT

A mobile laboratory exposure unit designed for inoculating human volunteers with bacterial and viral aerosols is described. Included are methods of generating aerosols of both small and large particles and of determining the size and concentration of particles. The aerosols contain one to 8,000 microorganisms per liter of air. Evaluation of the equipment includes tests for safety of personnel, determination of spray factors for predicting concentration of suspensions, comparison of samplers for more precise measurement of dilute aerosols (one organism per liter), comparisons between organism concentrations at sampling points and exposure ports, and between animal virulence assays.

Human volunteer exposures include (a) small-particle (one-micron-diameter) aerosols disseminated by a Collison atomizer; (b) large-particle (8- to 10.5-micron diameter) aerosols generated by a vibrating reed device; (c) respiratory vaccination with living vaccine cells of Pasteurella tularensis; and (d) small-particle aerosols containing Coxsackie A21 (Coe agent).

Results indicate the equipment is capable of generating aerosols that can be measured with a high degree of confidence.

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I. INTRODUCTION

Recent developments in vaccine production, coupled with a reliable chemotherapy for tularemia, have made it possible and safe to inoculate man subcutaneously with a disease-producing organism such as Pasteurella tularensis and determine the degree of protection afforded by the vaccine.[1,2] These findings led to the investigation of the efficacy of tularemia vaccines in men exposed to air-borne P. tularensis. Consequently, the United States Chemical Corps designed and constructed a mobile laboratory exposure unit by means of which human volunteers could be permitted to inhale precisely administered, safely and carefully controlled aerosols containing bacterial or viral organisms. The unit was made self-sustaining, so that it could be transported to any available source of human volunteers. Two of these sources have been the Ohio State Penitentiary in Columbus, Ohio,[3,4] and the House of Correction in Jessup, Maryland.[5]

This report describes the methods and aerosol apparatus used to induce infections by the respiratory route for further study, In addition, some of the achievements and developments that have been made possible by the precision obtained with this equipment are described.

II. DESCRIPTION OF THE EQUIPMENT

The mobile laboratory exposure unit was completed in 1957. It was designed using the basic semitrailer concept, in which the sides could be expanded to make more space available than in the conventional semitrailer. The basic unit when closed is 8 feet wide and 29 feet, 4 inches long and is mounted on rubber tires. A conventional tractor can be used to transport the unit (Figure 1).

A rack and pinion frame permits the sides to be expanded to an over-all width of 14 feet after the unit is adjusted to a level position with heavy-duty jacks. The expandable roof, floor, and end sections are mounted on heavy-duty piano-type hinges and are locked into place where they join the sides. Rubber gaskets along all the seams create an airtight seal. Figures 2,3, and 4 show the expanded trailer.

There are three main areas of operation: the laboratory, the exposure area, and the utility areas. A line drawing of these areas is shown in Figure 5.

The laboratory area (Figures 6 and 7) contains standard biological equipment such as a stainless steel work surface with two biological incubators and a refrigerator beneath; metal drawers and compartments are designed to remain closed during transport. There are also an electrical steam sterilizer, a stainless steel sink with an electric water heater underneath, a demineralizer, electric outlets, and sources of compressed air and vacuum. A Freon-tight cabinet constructed of plywood is mounted against the partition between the laboratory and exposure areas. This cabinet contains a modified Henderson apparatus[6] used to condition the biological aerosols for exposure of laboratory animals such as guinea pigs and monkeys, as well as for humans.* A control panel for operating the aerosol equipment is located at the rear of the laboratory area.

The area (Figure 8) that contains the equipment for exposing human volunteers, singly or in pairs, is a room 4 feet, 9 inches wide and 17 feet long. Molded rubber masks are mounted to exposure ports connected to the aerosol equipment in the laboratory area. An exposure control panel is mounted on the partition between the exposure and laboratory areas and contains measuring equipment such as air flow meters to determine volumes of air exhaled by the volunteers, electric timers to record length of exposures, and hand-controlled valves to initiate and terminate exposure of the volunteers.

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* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Figure 1. Mobile Unit Ready for Transport. (FD Neg C- 6994)

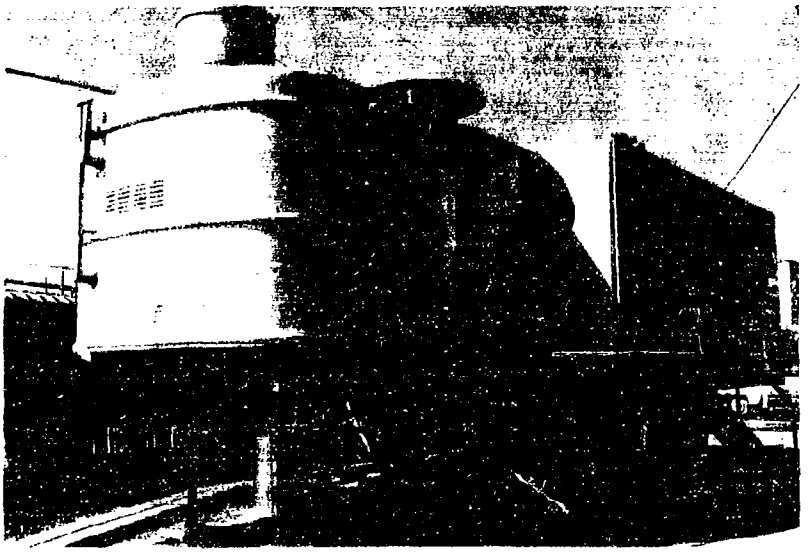

Figure 2. Side View of Mobile Unit Showing Utility and Exposure Area Entrances. (FD Neg C-2967)

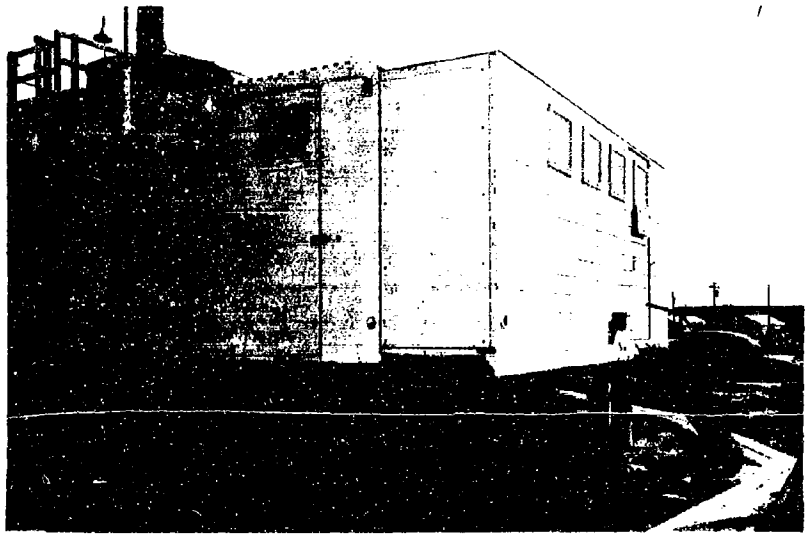

Figure 3. Side View of Mobile Unit Expanded, Showing Laboratory Entrance. (FD Neg C-2970)

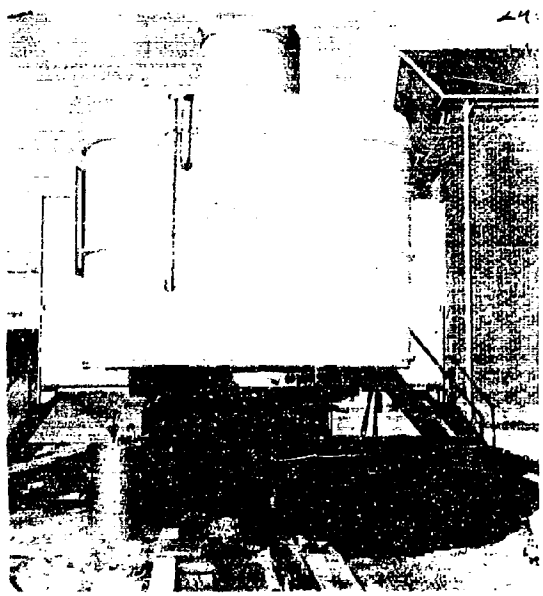

Figure 4. Front View of Mobile Unit in Expanded Position. (FD Neg C-2966)

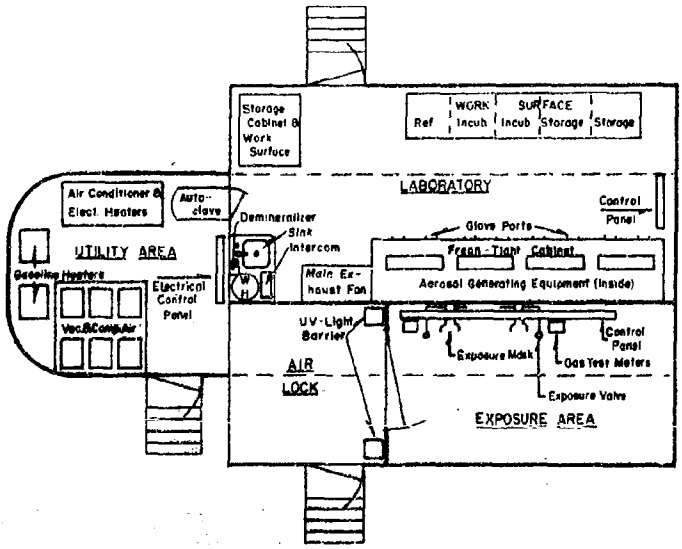

Figure 5. Line Drawing of the Three Main Areas of the Mobile Unit.

Figure 6. Rear View of Laboratory Showing Freon-Tight Cabinet, Control Panel, Work Surface (Refrigerator and Two B.O.D. Incubators Underneath). (FD Neg C-2961)

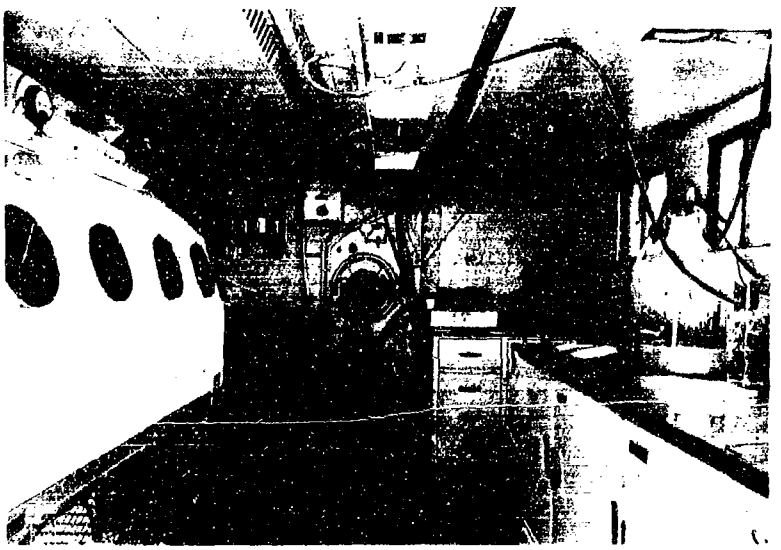

Figure 7. Front View of Laboratory Area Showing Intercom, Electric Steam Steriliser, Sink. (FD Neg C-2960)

Figure 8. Exposure Area Showing a Volunteer in the Process of a Simulated Exposure. (FD Neg C-2964)

The utility area, located at the front of the trailer, contains all of the equipment needed to generate compressed air and vacuum, electrical heaters, air conditioning equipment, supplemental gasoline-operated heaters for extreme cold-weather operation, and circulating coolant pumps to regulate aerosol temperatura (Figures 5 and 9). In addition, there is a stainless steel drum for waste disposal and a 100-gallon water tank underneath the framework.

For safety in the operation of laboratory and exposure equipment, all personnel wear plastic ventilated hoods that supply fresh filtered air. They also wear laboratory clothing such as jackets, trousers, gowns, rubber gloves, and rubber shoes, all of which may be sterilized before removal from the infectious areas of the trailer. In addition, the laboratory and exposure areas are designed with a relative negative pressure so that any escaping aerosol will be directed toward the aerosol tube; i.e., negative pressure is greatest inside the Henderson tube and decreases in the following order: Freon-tight cabinet, laboratory area, exposure area and, finally, the utility area, which is at normal atmospheric pressure. Filters in the exposure and laboratory areas, and in the aerosol equipment, maintain clean air supplies. Both laboratory and exposure areas and all of the aerosol and exposure equipment are decontaminated at the completion of an infectious operation by spraying a suitable disinfectant for at least 30 minutes.

An intercommunication system connects all three areas and the outside of the trailer for selective two-way communication. Microphones worn inside the plastic personnel hoods permit communication between any two areas of the trailer or the outside.

III. METHODS

A. AEROSOL GENERATION AND ASSESSMENT

A basic line drawing of the modified Henderson apparatus is shown in Figure 10. Aerosols are generated at one end and passed through a tube 6 inches in diameter and 7 feet long. The cloud is uniformly dispersed as it enters the tube by a 200- or 300-liter-per-minute flow of air humidified at 50 to 70 per cent. It is carried downstream at a constant rate under a negative pressure of 0.5 inch of water. The aerosol, therefore, is being generated at a constant rate and passes down the tube at a constant, regulated flow. By the time it has travelled six feet to the exposure and sampling ports, it is approximately ten seconds old. The aerosol exhaust is not recycled through the system as in the original Henderson apparatus; it is filtered, passed through ultraviolet light sterilizers, and filtered again before it is released to the open atmosphere. A negative-pressure regulator compensates for changes in air volume due to sampling and inhalations by volunteers.

Aerosols are generated by a Collison spray head[8] nebulizer for aerosol particles approximately one micron in diameter. Samples to determine cloud concentration are taken at sampling ports P_1 and P_2 (Figure 10) with such samplers as (a) Casella slit samplers, for dilute aerosol concentrations of 1 to 100 organisms per liter of aerosol; (b) all-glass impingers or modified versions of the Shipe impinger for concentrations greater than 100 organisms per liter; or (c) Cascade impactors for microscopic determination of the number and size of the aerosol particles. Descriptions and uses of these sampling devices are included in Public Health Monograph 60, "Sampling Microbiological Aerosols."[7]

Large-particle aerosols (8 to 20 microns in diameter) are generated by a vibrating reed[9] modified for use with infectious aerosols.

B. EXPOSURE OF ANIMALS

Guinea pigs are exposed to infectious aerosols by inserting only their noses directly into the air stream. The animals are placed in a cylindrical holder fitted to the exposure port.

Rhesus monkeys with plastic helmets fitted over their heads are exposed in a restraining chair.[9] The helmets are connected to the exposure ports and the aerosol is drawn over the animals' heads (Figure 10). The restraining chair orients the nose of the monkey directly in line with the exposure port; the rest of the body is protected from exposure by a rubber diaphragm fitted around the neck in the helmet; no anesthesia is required. Respiratory doses are based on the weight of the animal according to Guyton's factor.[10]

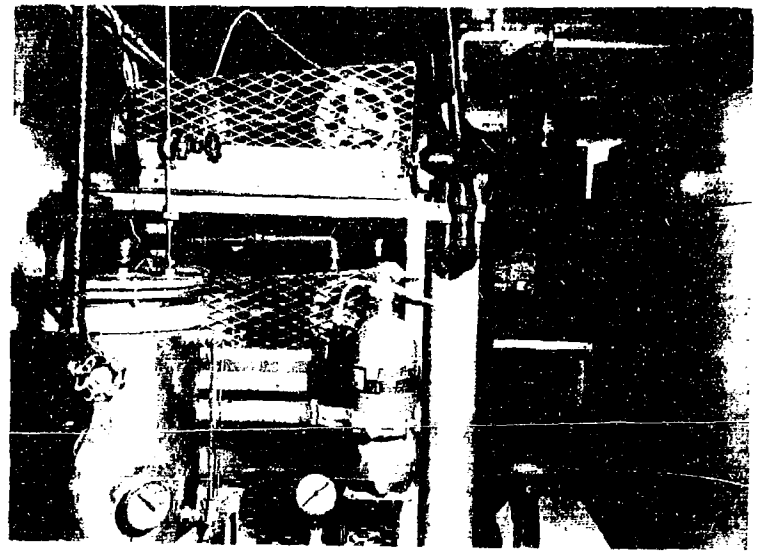

Figure 9. Interior of Utility Area Showing Some of the Operating Equipment. (PD Neg C-2965)

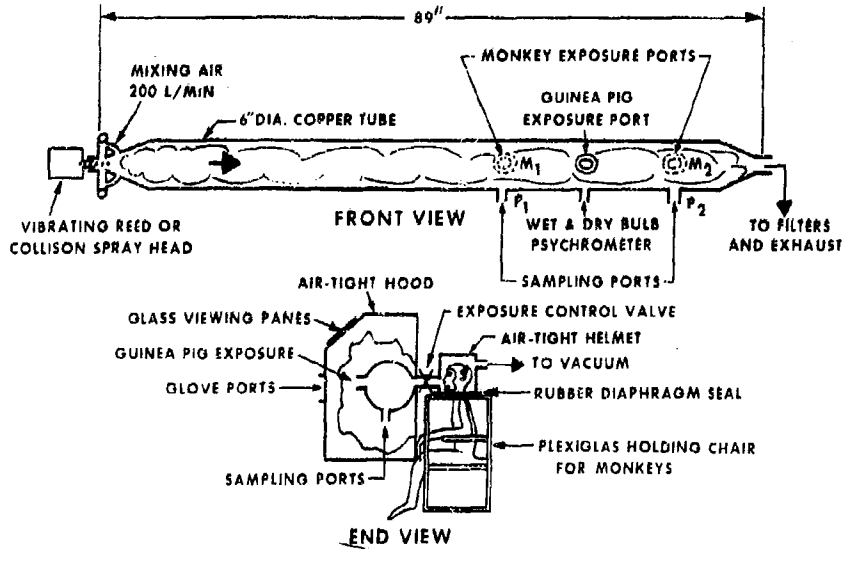

Figure 10. Basic Components of Modified Henderson Apparatus.

C. EXPOSURE OF HUMAN VOLUNTEERS

Prior to being exposed, human volunteers are familiarized with the equipment and methods by at least one, preferably two, simulated trials. They are instructed to place a rubber mouthpiece, which is mounted inside the exposure mask, between their lips and teeth. A monitor instructs each volunteer to inhale through the nose and exhale through the mouth. The exhaled air is passed through a gas meter that indicates the exhaled volume. The volunteer's respiratory volume is adjusted to approximately one liter of air per exhalation by visual observation of the gas test meters and instruction from the monitors. When each volunteer has adjusted to the required respiratory volume, the monitor opens a valve, permitting the aerosol from the Henderson apparatus to be inhaled. Opening and closing the valve activates an electric clock that automatically times the exposure. In addition to measuring the exhale volume, the monitors also count the number of respirations during each exposure. Thus the following determinations are made during a volunteer exposure: the concentration of the aerosol is determined from the Henderson apparatus, and the monitors record the exhaled volume, the number of respirations, and the time of exposure for each volunteer. Dosages are then calculated by multiplying the organisms per liter of aerosol by the volume of exhaled air.

IV. RESULTS

A. EVALUATION OF AEROSOL EQUIPMENT

The performance of the aerosol equipment was statistically evaluated. Important factors consisted of the following: (a) safety of the operating personnel and equipment, (b) the determination of the spray factor as a means of precision for obtaining predictable animal and human exposures, (c) comparison of various types of aerosol samplers, (d) comparisons between organism concentrations at the sampling ports (P_1 and P_2) and exposure ports (M_1 and M_2), and (e) virulence assays with guinea pigs and monkeys.

1. Safety Tests

To detect escape of aerosol from the equipment, we sprayed a suspension containing 1×10^8 Serratia marcescens organisms per milliliter of modified casein partial hydrolyzate diluted 1:1 with sterile distilled water into the modified Henderson apparatus. By means of air samples and surface swabbing of the cabinet, laboratory, and exposure areas, it was found that there was no escape of aerosol outside the Freon-tight cabinet. Recovery of organisms inside the cabinet indicated that aerosol escaped when samplers were removed from sampling ports and also when animals were removed from the animal exposure ports. The equipment was considered to be biologically safe for personnel, if they wore protective clothing and ventilated hoods and decontaminated all areas and equipment before leaving to shower.

2. Aerosol Sampler Comparison

A requirement for dilute concentrations of aerosols (one organism per liter of air) prevented the use of existing all-glass liquid impingers for sampling with any degree of confidence. The Casella slit sampler was adapted for these purposes and a series of comparison tests between the two samplers was made. Aerosols were generated at concentrations that could be confidently sampled by both devices. Twelve aerosols were generated and one-minute samples were taken at both sampling ports (P_1 and P_2) at 3, 8, 13, and 18 minutes after initiation of spraying. Both samplers were interchanged on the ports to give a quadratic interaction among samples taken at various ports by different samplers at different times. Data from these tests are shown in Table I and indicate a significant difference in recoveries between samplers. The ratio of slit sampler to impinger recoveries was 1.67 (95 per cent confidence limits 1.56 and 1.79). The Casella slit sampler was selected for more confident sampling of dilute aerosols of Pasteurella tularensis.

TABLE I. COMPARISON OF RECOVERIES [a] FROM ALL-GLASS IMPINGERS AND CASELLA SLIT SAMPLERS

Aerosol	Port	Sampler	Recovery (10^2 org/liter) at Time in Minutes				Averages	
			3	8	13	18	Impinger	Slit Sampler
1	1	1 [b]	1.60	1.30	1.04	1.78	1.43	
	2	S [c]	1.81	2.41	2.50	2.54		2.28
2	1	1	2.67	1.87	1.79	2.33	2.17	
	2	S	4.02	-	4.44	4.41		4.34
3	1	S	2.54	2.57	2.81	2.74		2.69
	2	1	1.25	2.30	1.44	1.86	1.71	
4	1	S	2.74	3.08	2.58	2.42		2.74
	2	1	1.04	1.68	1.91	2.49	1.78	
5	1	1	0.24	0.13	0.21	0.45	0.26	
	2	S	0.27	-	0.11	-		0.19
6	1	1	0.33	-	0.37	0.20	0.30	
	2	S	0.08	-	-	-		0.08
7	1	S	0.21	0.69	0.13	0.20		0.15
	2	1	0.21	0.10	0.11	0.36	0.20	
8	1	S	0.07	0.05	0.11	0.78		0.08
	2	1	0.13	0.15	0.17	-	0.15	
9	1	1	0.94	0.81	0.97	1.41	1.03	
	2	S	-	1.42	1.49	-		1.46
10	1	1	0.92	0.96	1.25	1.77	1.23	
	2	S	1.67	1.64	TMTC	TMTC		1.66
11	1	S	1.48	1.02	1.03	1.53		1.27
	2	1	0.57	0.82	0.57	0.83	0.70	
12	1	1	0.36	0.35	0.37	0.35	0.36	
	2	S	0.56	0.62	0.92	0.95		0.76
						AVERAGE	0.94	1.48

a. Organisms per liter
b. All-Glass Impinger
c. Casella Slit Sampler

3. Spray Factor Determinations

Spray factors[11] were determined by generating six replicate aerosols of P. tularensis at each of 14 predicted concentrations per liter of air, a total of 84 aerosols. Aerosols consisting of 0.5 to 100 organisms per liter of air were assayed with the Casella slit samplers; those with 100 to 10,000 organisms, with the all-glass impingers. Results of these spray-factor determinations are shown in Table II and indicate that the spray factor varied with the concentration of the suspension; i.e., as the spray concentration was changed, the spray factor did not remain constant, making it necessary to determine a spray factor before any exposure trial in order to present a more precise dose.

4. Sampling And Exposure Port Comparison

Organisms recovered from the mask ports (M_1 and M_2) were compared with organisms recovered from the sampling ports (P_1 and P_2). Eight replicate aerosols containing approximately one organism per liter of air were generated and slit samples were pulled from both mask and sampling ports at 3, 8, 13, and 18 minutes after initiation of spraying. Results of these tests, shown in Table III, indicated no significant difference between the corresponding masks and ports; the variation of samples within runs was 29 per cent and a single future observation may vary up to 3.34-fold from a predicted or expected value (3.34 is the 95 per cent confidence limit factor) for this concentration of aerosol.

5. Animal Virulence Assay

Guinea pigs (Hartley strain) and Macaca mulatta were exposed to several aerosol concentrations of P. tularensis to determine inhaled doses required to produce infection, using the aerosol equipment of the mobile unit. Guinea pigs developed infection from inhalation of only one viable organism (LD_{50} of 3 inhaled organisms). The respiratory LD_{50} for the monkey was about 17 inhaled organisms.

B. HUMAN VOLUNTEER EXPOSURES

Human volunteers have been exposed to one-micron-diameter particles containing P. tularensis for immunization studies[3-5] and to similar sized aerosol particles containing Coxsackie A-21 (Coe agent) for respiratory infectivity studies.[12] The Coe agent investigations were made in cooperation with Dr. Vernon Knight, Laboratory of Clinical Investigations, National Institutes of Health.

Volunteers have also been exposed to aerosols containing the living vaccine strain of P. tularensis to determine the effect of the route of immunization, and to large-particle aerosols (8-, 9-, and 10-micron diameter) containing P. tularensis to determine the effect of large particles on respiratory infections. These results are intended for future publication. Similar studies are planned for exposing volunteers to large aerosol particles containing Cosackie A-21.

Results of tests involving aerosol immunization of guinea pigs and rhesus monkeys, reported by Eigelsbach et al,[13] indicate that a greater degree of protection against either subcutaneous or respiratory challenge is afforded by immunization via the respiratory route than by the subcutaneous route. To date, one notable advantage to the respiratory route of immunization is the absence of secondary side effects. Volunteers notice no appreciable effects due to inhalation of the vaccine cells.

TABLE II. EFFECT OF CONCENTRATION OF SPRAY SUSPENSION UPON SPRAY FACTOR

Spray Suspension, org/ml	Spray Factor	org/liter
2.58×10^{8}	1.5×10^{-8}	10,000
1.5×10^{8}	1.3×10^{-8}	5,000
7.2×10^{7}	1.0×10^{-8}	2,000
4.1×10^{7}	9.1×10^{-7}	1,000
2.3×10^{7}	8.1×10^{-7}	500
1.0×10^{7}	7.2×10^{-7}	200
5.5×10^{6}	6.8×10^{-7}	100
2.8×10^{6}	6.6×10^{-7}	50
1.1×10^{6}	6.6×10^{-7}	20
5.2×10^{5}	7.1×10^{-7}	10
2.4×10^{5}	7.8×10^{-7}	5
7.6×10^{4}	9.8×10^{-7}	2
3.0×10^{4}	1.3×10^{-8}	1
1.0×10^{4}	1.8×10^{-8}	0.5

TABLE III. COMPARISON OF ORGANISMS RECOVERED FROM THE MASK PORTS AND SAMPLING PORTS

Aerosol	Port	Recovery (org/liter) at Time in Minutes				Averages	
		3	8	13	18	Mask	Sample
1	M [a]	0.70	1.52	0.66	-	0.84	
	S [b]	-	0.98	0.72	0.75		0.68
2	M	-	0.98	0.82	1.41	1.05	
	S	-	0.83	0.98	0.79		0.87
3	M	3.45	-	6.15	6.62	5.80	
	S	4.18	6.11	4.49	6.37		5.28
4	M	3.52	4.03	6.70	4.94	4.63	
	S	3.47	4.37	7.39	5.24		5.13
5	M	12.62	18.23	17.17	15.96	15.9	
	S	13.28	16.37	15.69	16.45		15.4
6	M	10.49	11.09	11.50	13.92	12.2	
	S	6.15	7.16	10.98	13.58		9.5
7	M	5.84	5.37	4.66	5.52	5.4	
	S	2.56	4.83	4.86	3.13		3.8
8	M	4.71	5.69	5.05	2.41	4.7	
	S	4.18	-	-	3.88		4.0
				AVERAGE		6.32	5.58

a. Mask Port
b. Sampling Port

V. DISCUSSION

Reliability of the assessment of aerosols generated in the mobile laboratory exposure unit has made it possible to present as few as one, and as many as 8,000, organisms of P. tularensis per liter of air to human volunteers. Predicted inhaled doses have been well within the 3.34 factor of variation previously described.

Production of homogeneous aerosol particles by the vibrating reed has made it possible to investigate air-borne infectivity with a much more refined method.

Selected aerosols of homogeneous particle size can be used in combination with various types of upper respiratory viruses to investigate many areas of human respiratory diseases. For example, the particles present in an ordinary sneeze could be separated into various sizes to determine which are the important sites of infection. Nasal inhalation versus oral inhalation could also be compared and the effect of humidity on respiratory infections in humans could be investigated.

Finally, the route of respiratory immunization could be investigated with a vast number of different vaccines to improve man's protection against diseases.

VI. CONCLUSIONS

The aerosol apparatus and equipment of the mobile laboratory exposure unit have proved to be an excellent means of testing the efficacy of vaccines in human volunteers. The high degree of efficiency and control, and the selection of particle size, have made it possible to make quantitative determinations of respiratory dosages. The use of living vaccines has permitted immunization of humans via the respiratory route without subjecting them to secondary side effects encountered with subcutaneous immunization.

VII. SUMMARY

A mobile laboratory exposure unit designed to test efficacy of bacterial vaccines and to determine more accurately bacterial and viral infections in human volunteers via the respiratory route was designed and built. Tests were made to determine the performance of the aerosol equipment and improve sampling methods for measuring aerosols containing as few as one organism per liter of air with a high degree of confidence.

Tests performed included safety of operations, determination of a spray factor for obtaining predictable dosages, comparison of aerosol samplers for dilute aerosol concentrations, comparison of the recoveries at the sampling and exposure ports, and virulence assays with guinea pigs.

Methods of exposing animals and human volunteers to the aerosols are described.

Tests performed indicate that the mobile laboratory exposure unit is safe for operating personnel provided that they wear plastic ventillated hoods, protective clothing, and shower when finished. It is necessary to make preliminary tests to determine the spray factor before any exposure trial in order to present a reliable dose. Casella slit samplers were shown to be more accurate for sampling dilute aerosols than liquid impingers. There was no significant difference between recoveries of the small-particle aerosols at the sampling and exposure ports.

The high degree of efficiency and the use of living vaccine cells of P. tularensis have made it possible to determine respiratory dosages quantitatively and to immunize humans via the respiratory route.

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